obscura. The results of the experiments with hybrids are consistent with this hypothesis (table 1). Males of *D. persimilis* inseminate a higher percentage of the hybrid than of their own females. The isolation index[1] is negative in both crosses (−0.15, −0.32). The greater activity of the hybrid females is apparently more than sufficient with males of *D. persimilis* to compensate for their genetic inferiority in regard to factors one and two. The greater activity of the hybrid females is not quite sufficient in tests with the males of *D. pseudoobscura* to overcome the adverse influence of factors one and two. The isolation index remains positive (+0.11, +0.42). Still, the discrimination of the *pseudoobscura* males against hybrid females is much slighter than against *persimilis* females. At best (with *persimilis* ♀ × *pseudoobscura* ♂ hybrids), only twice as many of their own females are inseminated as against ten times as many in the control experiment.

The relative desirability of the hybrid females is a puzzling fact, considering the wide overlap of the two species in nature. There would seem to be an apparent opportunity for a good deal of introgressive hybridization. The factors that keep this potential danger in check need further investigation.

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*INHERITED DIFFERENCES IN SENSITIVITY TO RADIATION IN ESCHERICHIA COLI**

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The study of spontaneous and radiation-induced mutations is at present our best approach to the investigation of genetic mechanisms in bacteria. Mutations involving resistance to destructive agents (bacteriophage,[1] penicillin[2]) are especially suitable for genetic analysis, since resistant mutants can easily be detected in bacterial cultures. This preliminary report concerns a mutation in *Escherichia coli* leading to resistance to both ultraviolet radiation and x-rays, which was detected by exposing samples from normal cultures to high doses of radiation.

Most investigators of the effects of ultraviolet radiation on bacteria have considered the population within a strain to be fundamentally uniform in sensitivity. Most of the differences found seem to depend upon transient

physiological factors (age of cells, conditions of culture). Rentschler, Nagy and Mouromoff,[3] however, reported genetic differences in sensitivity to ultraviolet in *E. coli*, and Hollaender[4] noted that, in a population of *E. coli*, one bacterium in a million could survive irradiation with very high doses.

Material and Methods.—Strain *B* of *Escherichia coli* was used throughout these experiments. A stock slant was established from a single-colony isolation at the start, and was subcultured every two months. Cultures inoculated with samples from the same slant were used for all comparable experiments.

Difco nutrient broth and nutrient agar were the media used.

TABLE 1

SENSITIVITY TO ULTRAVIOLET OF BACTERIA SURVIVING IRRADIATION WITH A HIGH DOSE OF ULTRAVIOLET

Origin of control cultures: single colonies from a non-irradiated plate seeded with bacteria from strain *B*. Origin of experimental cultures: single-colony survivors from plates seeded with bacteria from strain *B*, and irradiated with a dose of 1000 ergs/mm.^{2}.

	TREATMENT: 90 ergs/mm.^{2}			TREATMENT: 550 ergs/mm.^{2}		
	CELLS PER	SURVIVORS		CELLS PER	SURVIVORS	
CULTURE	SAMPLE	NUMBER	PER CENT	SAMPLE	NUMBER	PER CENT
Control—1	520	18	3.5	1040	4	0.4
Control—2	481	26	5.4	962	5	0.5
Control—3	456	13	2.9	912	8	0.9
Control—4	509	30	5.9	1018	2	0.2
Experimental—1	455	422	92.9	890	336	37.7
Experimental—2	520	507	97.5	1040	416	40.0
Experimental—3	490	482	98.3	980	424	43.2
Experimental—4	512	473	92.3	1024	391	38.1

The source of ultraviolet radiation was a General Electric low-pressure mercury-vapor lamp, emitting unfiltered radiation primarily of wavelength 2537 A. Doses are expressed in ergs per mm.^{2}, but these values are approximate, since an indirect biological method of calibration was used.

Bacteria to be irradiated with ultraviolet were taken from 24-hour broth cultures and diluted quantitatively in broth. Measured samples were spread evenly on the surface of nutrient-agar Petri dishes with a sterile glass rod. The plates were then exposed to the radiation, and colony counts were made after 24 hours of incubation. Survival was measured by comparison with non-irradiated control plates.

Irradiation with x-rays was conducted at Memorial Hospital in New York City, through the courtesy of Mr. L. D. Marinelli, and with the assistance of Miss E. Focht. The source emitted unfiltered rays of 180 kv., with an intensity of 2050 roentgens per minute.

Bacteria to be irradiated with x-rays were taken from undiluted 24-hour aerated broth cultures, and were exposed in small, thin-walled glass tubes.

Measured dilutions were made after irradiation, and were plated out. Colony counts were made after incubation, and compared with non-irradiated controls.

Assays to determine the titre of liquid cultures were made by plating measured dilutions and making colony counts.

Experimental Results.—Derivation of the Resistant Strain: A sample of about 5×10^4 bacteria from a culture of *B* was irradiated with a dose of 1000 ergs per mm.^{2}. After 24 hours of incubation, 4 colonies had developed, indicating that only 4 bacteria had survived the irradiation. These colonies were inoculated separately into broth, and samples from the resulting cultures were irradiated to determine their sensitivity to 2 test doses of ultraviolet. Four control cultures, started from single colonies on a non-irradiated plate, were irradiated with the same doses, and the survival of the 2 sets of cultures was compared. Table 1 gives the results, which indicate that all 4 survivors were characterized by markedly greater resistance to ultraviolet than the normal strain.

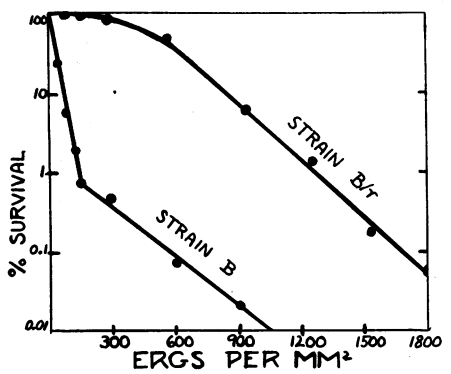

Figure 1

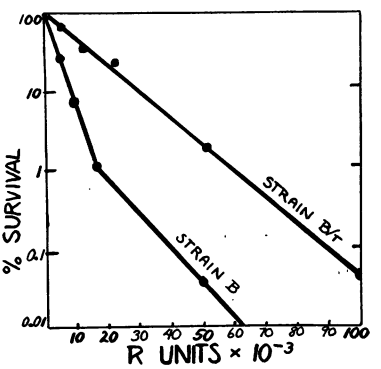

Figure 2

Figure 1. Ultraviolet Survival Curves of Strains *B* and *B/r*.
Figure 2. X-Ray Survival Curves of Strains *B* and *B/r*.

One of these 4 resistant cultures (No. 1) was established on an agar slant to serve as a stock-resistant strain for further study. This strain will be referred to as strain *B/r* (*B* resistant to radiation).

Properties of the Resistant Strain: (1) *Stability of Strain B/r.*—The resistant strain *B/r* has been carried through over 50 subcultures in broth, and for a period of 18 months on agar, with no change in ultraviolet sensitivity or in other observed properties. Ultraviolet resistance, therefore, may be considered a stable, heritable character.

(2) *Sensitivity of Strain B/r to Ultraviolet.*—The curves of survival of strains *B* and *B/r* as a function of ultraviolet dose are given in figure 1. Single-colony survivors were isolated from various points along the curve

of strain *B*, and were tested for sensitivity to ultraviolet. Only the two levels of sensitivity represented by strains *B* and *B/r* were observed. No intermediate resistance, and no resistance greater than that of *B/r* were detected.

(3) *Sensitivity of Strain B/r to X-rays.*—Figure 2 gives survival curves of strains *B* and *B/r* with x-rays. It is evident that strain *B/r* is relatively resistant to x-rays, as well as to ultraviolet radiation.

The survival curves of the normal strain with both ultraviolet and x-rays show a change in slope at about 1% survival. The change in killing rate at this point can be partially, but not entirely, explained by the presence of resistant bacteria in the normal sample.

(4) *Growth Rate of Strain B/r.*—Growth curves of strains *B* and *B/r* in broth at 37°C. were compared. The lag phase of *B/r* was found to be about 25% shorter than that of the normal strain. The generation time was the same for the two strains (19 minutes).

A Technique for the Quantitative Detection of Radiation-Resistant Bacteria in Samples from Normal Cultures: Before the genetic basis of resistance to radiation could be determined, it was necessary to overcome a methodological obstacle. The difficulty was based on the fact that resistance to radiation is relative, rather than absolute.

If spontaneous mutation is wholly or partially responsible for the change in sensitivity, a normal culture must contain, prior to irradiation, a certain proportion of resistant cells. A glance at the survival curves (Figs. 1 and 2) will show that a dose high enough to eliminate all or most of the sensitive bacteria will also eliminate most of the resistant cells. Treatment with a high dose of ultraviolet, therefore, will permit the recovery of only a small fraction of the resistant bacteria originally present in the sample.

Since an accurate determination of the number of resistants present in various samples is necessary for any quantitative study of the origin of the resistant bacteria, a method was developed whereby all the resistant bacteria in a normal sample could be detected.

The first clue to this technique was obtained by observing ultraviolet-irradiated bacteria of strains *B* and *B/r* under the microscope. It is well known that irradiated bacteria, within a certain range of doses, grow in length for several hours before dividing, forming snake-like filaments sometimes hundreds of times their normal length.

Samples of *B* and *B/r* were irradiated with a dose of 50 ergs per mm.^{2}, which permits 100% survival of resistant bacteria and reduces the number of sensitive bacteria to about 10%. The irradiated plates were incubated, and the bacteria were examined at intervals under the microscope.

After 3 hours, the irradiated cells of strain *B* were extremely elongated and still undivided. Division of the resistant cells, however, was not inhibited by this dose, and after 3 hours each originally present resistant

bacterium had given rise to a microcolony of about 100 cells of normal length.

The plates were given a second irradiation of 700 ergs per mm.^{2}, 3 hours after the first. On the one hand, this treatment should reduce each resistant microcolony to about 10 cells. If at least one cell in each resistant microcolony survives this second treatment, a visible colony for every originally present resistant bacterium will form. On the other hand, the second irradiation should eliminate all the sensitive survivors of the first treatment, if the number of bacteria in the original sample is not more than 2×10^4, and if the elongated cells resulting from the first irradiation behave like single bacteria rather than like chains of bacteria in their sensitivity to ultraviolet. Lea, Haines and Coulson[5] found that this was true of long forms produced by gamma rays.

TABLE 2

RELIABILITY OF DOUBLE-IRRADIATION TECHNIQUE FOR SELECTIVE RECOVERY OF RADIATION-RESISTANT BACTERIA IN MIXTURE WITH SENSITIVE BACTERIA

Double-irradiation technique: first dose of 50 ergs/mm.^{2} followed by 3 hours of incubation and second dose of 700 ergs/mm.^{2}.

		INITIAL NUMBER OF CELLS PER SAMPLE		COLONY COUNT AFTER TREATMENT AND INCUBATION	
CULTURE	SAMPLE	*B*	*B/r*	SENSITIVE	RESISTANT
Normal (*B*)	1			0	1
	2			0	0
	3			0	0
	4			0	1
		Av. 11,200	...	...	...
Resistant	1			0	114
(*B/r*)	2			0	91
	3			0	120
	4			0	130
		...	Av. 111 ± 9.0	...	Av. 113.7 ± 16.5
Mixture	1			0	94
(*B* + *B/r*)	2			0	112
	3			0	106
	4			0	96
		Av. 9,400	Av. 106 ± 8.5	...	Av. 102.0 ± 8.5

Table 2 gives results of an experiment to determine the validity of this technique. Samples of *B*, *B/r* and of a mixture of the two strains were given this double-irradiation treatment, and the results, which were repeatedly confirmed, indicate that it is effective in permitting selective survival of resistant bacteria.

The technique can be modified to determine the number of resistant bacteria in samples as large as 10^7, by extending the time of incubation between irradiations up to 5 hours, and increasing the second dose up to 1800 ergs per mm.^{2}. When large samples are used, however, sensitive

bacteria occasionally survive, probably because of screening due to the dense network of elongated cells which develops after the first irradiation. When samples of about 10^6 or more bacteria are used, it is necessary, therefore, to determine the sensitivity of each surviving colony. This can be done rapidly by suspending bacteria from the colony to be tested in a drop of broth, spreading the suspension on agar and irradiating with a dose of 50 ergs per mm.^{2}. After 3 hours of incubation, the bacteria are examined under the microscope. If the colony consisted of sensitive bacteria, the microscope will reveal thread-like elongated cells. If the colony consisted of resistant bacteria, microcolonies of about 100 cells of normal length will be observed.

Origin of the Change from Sensitivity to Resistance: It is important to establish the mode of origin of hereditary variations in bacteria. In the case of radiation-resistance the problem is of particular interest, since ultraviolet radiation and x-rays are known to be effective in inducing mutations.

The possible modes of origin may be considered as follows: (1) The change to increased resistance is induced by the radiation in a certain number of cells in an initially homogeneous population; or (2) the change is a spontaneously occurring mutation, and prior to the treatment the culture contains a certain number of resistant mutants. In this case (*a*) the radiation acts merely as a selective agent, or (*b*) the radiation acts as a selective agent, but also acts as an inducing agent, increasing the rate of mutation to resistance.

The method used to test these hypotheses was that developed by Luria and Delbrück[1] and applied by them and by Demerec[2] to the study of bacterial mutations. The method involves the following considerations:

If the change is entirely induced by the radiation, the number of resistant bacteria obtained from a sample will depend upon the probability that an induced change will occur in any bacterium. This probability should be the same for all bacteria under similar physiological conditions. Therefore, the number of resistants in samples from a series of similar, independent cultures should show fluctuations no greater than those shown by the number of resistants in a series of samples from a single culture. These fluctuations should be due only to sampling error, and in both cases the distribution of the number of resistants should constitute a Poisson series, with the variance approximately equal to the mean.

If the change is a spontaneous mutation, the number of resistants obtained from a given sample depends upon: (1) the probability that any bacterium will mutate during its lifetime, and (2) the time of occurrence of mutations during the growth of the culture, since all bacteria descended from mutated cells will be resistant. In this case, the number of resistants in samples from a series of similar, independent cultures should show large

fluctuations (see Luria and Delbrück[1]), and the variance should be significantly higher than the mean.

Table 3 gives results of experiments to determine the number of resistant bacteria in samples from a series of similar, independent cultures and in samples from a single culture. Every culture was started with an inoculum of about 20 cells from the normal strain, in a volume of 1 ml. of broth. The final titre of the cultures in any series differed by not more than 12%.

TABLE 3

NUMBER OF RESISTANT BACTERIA IN SAMPLES FROM INDEPENDENT CULTURES, AND IN SAMPLES FROM SINGLE CULTURES

Number of resistant bacteria determined by double-irradiation technique: first dose of 50 ergs/mm.^{2} followed by 5 hours of incubation, and second dose of 1500 ergs/mm.^{2}.

	SAMPLES FROM INDEPENDENT CULTURES				SAMPLES FROM SINGLE CULTURES		
EXPT. NO.	1	2	3		1	2	3
AV. NO. CELLS PER SAMPLE CULTURE	1×10^6	1.1×10^6	9.5×10^5		9.7×10^5	1×10^6	1.2×10^6
NO.				SAMPLE NO.			
1	0	4	5	1	8	0	13
2	12	8	13	2	10	1	9
3	8	15	61	3	5	3	11
4	8	12	10	4	9	1	14
5	19	0	1	5	7	0	9
6	98	14	12	6	9	0	8
7	7	1	2	7	15	2	12
8	5	76	8	8	8	1	13
9	14	11	0	9	6	4	7
10	24	42	13	10	16	1	15
11	..	..	9	11	..	..	8
12	..	..	7	12	..	..	8
13	..	..	18	13	..	..	14
14	..	..	0	14	..	..	9
15	..	..	11	15	..	..	13
16	..	..	8	16	..	..	6
17	..	..	116	17	..	..	11
18	..	..	12	18	..	..	7
19	..	..	10	19	..	..	10
20	..	..	6	20	..	..	18
Average	19.5	18.3	16.1	..	9.3	1.3	10.8
Variance	764.7	574.7	1509.8	..	12.9	1.8	6.9
χ^2	373.3	279.6	844.0	..	12.5	11.6	12.2
P	..	..	..	..	0.1–0.2	0.2–0.3	0.8–0.9

In all three experiments, the number of resistants in samples from a single culture shows fluctuations satisfactorily accounted for by sampling errors. This indicates that the method of plating and irradiating does not introduce fluctuations beyond those expected on the basis of sampling.

The number of resistants in samples from a series of independent cultures,

in all three experiments, shows fluctuations much greater than can be accounted for by sampling errors. The variance is significantly higher than the mean in every case, and the fluctuations are of the type to be expected according to the hypothesis of spontaneous mutation.

The possibility remains that, while radiation-resistance occurs as a spontaneous mutation, the treatment used to detect these mutants induces the change in an additional number of sensitive bacteria. Indeed, such an effect should be expected on the basis of the known power of ultraviolet to induce mutations. Under the conditions of the experiment, however, it is not likely that induced mutations could be detected. The double-irradiation technique permits the survival only of bacteria that are resistant at the time of the treatment—no sensitive cell has a chance to survive. Therefore, sensitive bacteria in which resistance is induced during the treatment will survive only if they become phenotypically resistant *immediately*. The following evidence suggests that induced mutations, if they occur, are not detected by the double-irradiation technique. The doses used in this treatment, depending upon the initial number of bacteria in the sample, vary from 700 to 1800 ergs per mm.^{2} If the number of mutants in several samples from the same culture is determined, using doses throughout this range, the proportion of mutants in the various samples is found to be the same. If these mutants arise by induction, their number should be proportional to the dose.

The Mutation Rate: It is possible to estimate the rate of bacterial mutations from experiments of the type described above by solving the following equation: $r = aN_t \ln (CaN_t)$ (for derivation see Luria and Delbrück[1]), where r is the experimental average of the number of mutants in a series of similar cultures, N_t is the number of bacteria at the time of observation, C is the number of cultures and a is the mutation rate.

Luria and Delbrück have plotted a series of curves relating the observed values of r to aN_t for various values of C. The estimated mutation rate for radiation-resistance, obtained from these curves, is about 10^{-5} mutations per bacterium per generation.

Discussion.—The evidence presented indicates that the heightened resistance to radiation exhibited by bacteria of strain B/r is the expression of a mutation which occurs spontaneously in cultures of strain B. No critical evidence is available which precludes the possibility that the observed change in sensitivity may be produced by different mutations. However, unrelated resistant strains, each isolated from a different single-cell culture of strain B, exhibit similar sensitivity to ultraviolet and to x-rays, similar growth rates and growth requirements, similar colony characteristics and similar patterns of individual cell growth and division after irradiation. If different mutations give rise to radiation-resistance, their effects must be identical in so far as these properties are concerned.

The fact that *B/r* is resistant to both ultraviolet and x-rays suggests that the mutation affects a process which is involved in the lethal action of both types of radiation, unless it is assumed that the mutation involves more than one fundamental change.

The mechanism of the lethal action of radiations on bacteria is so poorly understood that it is difficult to speculate about the changes that might be responsible for the heightened resistance of the mutant *B/r*. The survival curves (Figs. 1 and 2), if interpreted on the basis of the hit theory of radiation effects, suggest a possible difference between sensitive and resistant bacteria. The ultraviolet and x-ray survival curves of strain *B* are both exponential "one-hit-to-kill" curves. The ultraviolet survival curve of strain *B/r* may be interpreted as a multiple-hit curve, and suggests that this strain differs from the normal strain in possessing multiple centers of lethal action. The exponential x-ray curve of *B/r* seems to contradict this idea, but could be explained by the assumption that a single x-ray "hit" may consist of several ionizations, and may produce more than one change, a possibility stressed recently by Lea and Catcheside.[6]

Another clue to the mechanism of resistance is obtained from the microscopic observation of ultraviolet-irradiated bacteria of strains *B* and *B/r*. The radiation exerts a strong inhibitory effect on cell division in sensitive bacteria, resulting in the production of long forms. The division mechanism of resistant bacteria is not appreciably inhibited by the same dose that delays division in strain *B* for several hours, indicating that the resistance to radiation is a specific resistance of the division mechanism. The hardier division mechanism of strain *B/r* may be seen as the cause of the shorter lag phase of that strain, which may or may not be related to its radiation-resistance.

Lea, Haines and Coulson[5] have considered the lethal action and the inhibition of division as independent effects of gamma rays. Bacteria of strain *B/r* differ from sensitive bacteria in degree of susceptibility to both the lethal action and the division-inhibiting action of ultraviolet. Again, unless two basic differences between the two strains are assumed, it seems likely that the power to inhibit division and the lethal effect, at least of ultraviolet, are directly related.

Summary.—(1) Strain *B* of *Escherichia coli* yields variants which are characterized by resistance to both ultraviolet radiation and x-rays, and which can be detected by the selective action of these radiations.

(2) Resistance to ultraviolet radiation and x-rays is a stable, heritable character.

(3) A technique is described whereby the number of resistant bacteria in samples from normal cultures can be determined accurately.

(4) The change from sensitivity to resistance is a spontaneous muta-

tion occurring in normal cultures at a rate of about 1×10^{-5} mutations per bacterium per generation.

(5) Doses of ultraviolet that inhibit cell division in sensitive bacteria for several hours, resulting in the production of elongated cells, do not appreciably inhibit division in resistant bacteria.

(6) Curves of survival of sensitive and resistant bacteria as a function of ultraviolet and x-ray doses are compared.

(7) Interpretations of certain features of the results are discussed.

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A NEW GENE THEORY AND AN EXPLANATION OF THE PHENOMENON OF DOMINANCE TO MENDELIAN SEGREGATION OF THE CYTOGENE

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The chromosomes have often been called the "bearers" of the hereditary factors and the terms "gene" and "locus" have been considered synonymous. This terminology was prophetic of the real nature of the hereditary mechanism. The experiments presented herewith show that the locus, which I propose to call the *chromogene*, is simply a place of attachment for the cytogene. This view corroborates the principle of the duality of the gene originally announced by Sonneborn,[3] with this significant difference: the cytogene[1, 2, 4] is an entity capable of self-duplication in the cytoplasm. Sonneborn's kappa substance only multiplies in the macronucleus; it does not multiply in the cytoplasm. The cytogene may be transmitted from parent to offspring either by being held at the dominant chromogene in the absence of its specific substrate, or by continued multiplication in the cytoplasm of cells containing the recessive chromogene if the specific substrate is present. A cell containing the dominant (fermenting) gene is capable of fermenting a specific carbohydrate, thus indicating that it is